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Anti-inflammatory activity of leaves of *Jatropha gossypifolia* L. by hrbc membrane stabilization method

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ABSTRACT

Object: To evaluate the anti inflammatory activity of leaves extracts of *Jatropha gossypifolia* (*J. gossypifolia*) L. **Methods:** The plant *J. gossypifolia* L. (Eurphorbiaceae) is known as belly ache bush. The plant originated from Brazil and it is now cultivated in tropical countries throughout the world. The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. The young stem of the plant is used as tooth brush as well as to clean tongue in the treatment thrush. The tuber of the plant grinded into a paste is also locally used in the treatment of hemorrhoids. The present study aimed to evaluate the anti inflammatory activity of aqueous and alcoholic extract of *J. gossypifolia* leaves by *in vitro* HRBC membrane stabilization method. **Results:** The *in vitro* method showed significant anti inflammatory property of different extracts tested. **Conclusion:** The aqueous extract at a concentration of 200 μg/mL showed significant activity when compared with the standard drug Diclofenac sodium.

1. Introduction

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses[1]. Inspite of our dependence on local medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently complied an inventory of medicinal plants listing over 20 000 species. The family Euphorbiaceous consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phytochemical constituents. The main action of anti inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of Hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti inflammatory property of various extracts of *Jatropha gossypifolia* (*J. gossypifolia*) L. Thus human blood cell membrane stabilization (HRBC method[2]) has been used as a method in estimating the anti inflammatory property. In certain parts the leaf of this plant was traditionally used in the treatment of hemorrhoids. The present study aimed to authenticate the traditional anti inflammatory activity of this species by *in vitro* anti inflammatory screening.

2. Materials and methods

2.1. Preparation of extracts

The plant material was collected from the plant *J. gossypifolia* L. which are collected during the month

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of December at Potharlanka near Repalle, Guntur (Dist) of Andhra Pradesh. Then it was authentified by Dr. SM. Khasim, professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur. The shade dried leaves were powdered and extracted with soxhlet apparatus using ethanol (yield 5.8%) and distilled water (yield 4.2%) separately. Both the extracts were evaporated to dryness. The samples were prepared by suspending the residues in hot water and used for anti-inflammatory study.

2.2. Chemicals and instruments

All chemicals used in the study were of analytical grade. Dextrose, Sodium citrate, Citric acid, Sodium chloride, Sodium hydroxide and Dihydrogen phosphate was purchased from SD fine chemicals, Mumbai. Reference standard Diclofenac was obtained from Cipla Ltd, Bangalore. Shimadzu 1701 UV-Visible spectrophotometer was used for the estimation of anti inflammatory activity.

2.3. Acute oral toxicity

The acute toxicity for the JGE was conducted in rats with body weight 200-250 g as per the prescribed guidelines. Ten animals of either sex were used in each group of extract. Their weights were recorded before beginning the study. They were administered with bolus dose of the JGE (500, 1 000, 1 500, 2 000 mg/kg) per oral and observed over 14 d for mortality and physical or behavioral changes[3]. The study was approved by IAEC of Vignan Pharmacy College, Vadlamudi. (1499/PO/a/11/CPCSEA).

2.4. In vitro anti - inflammatory activity

The anti inflammatory activity of leaf extract of *Jatropha gossypifolia* Linn was determined by HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium chloride in water). The blood was centrifuged at 300 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) & 10% v/v suspension was made with isosaline.

The assay mixture contained the drug (concentration as mentioned in Table 1). 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL of hyposaline (0.36%) % 0.5 mL of HRBC suspension. Diclofenac was used as the reference drug. Instead of hyposaline, 2 mL of distilled water was used as control. All the assay mixtures were collected at 37 ℃ for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using colorimeter at 560 nm. The percentage heamolysis was calculated by assuming the heamolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula[4-8].

$$\% \text{ protection} = 100 - \frac{\text{O.D of drug treated sample}}{\text{O.D of control}} \times 100$$

2.5. Statistical analysis

Statistical analysis was done using one way ANNOVA followed by Dunnets test. P values greater than > 0.05 were considered as significantC

3. Results

3.1. Acute oral toxicity study

The extracts of *J. gossypifolia* L. did not show any sign of toxicity up to 2 000 mg/mL body weight and hence it was considered to be safe.

3.2. In vitro anti inflammatory activity

J. gossypifolia L. ethanolic and aqueous extracts at different concentrations (100, 200 μg/mL) showed significant stabilization towards HRBC membrane. The percentage protection of aqueous extract at concentration 200 μg/mL was higher than that of concentrations. However the percentage protection was found to be increased at higher concentrations. The results were tabulated in Table 1.

Table 1
% Protection of heamolysis from HRBC membrane stabilization method.

Concentration (μg/mL)	Activity (Prevention of lysis in %)		
	Diclofenac	Alcoholic extract	Aqueous extract
100	9.56	19.1	14.2
200	-	1.6	56.8

Aqueous extract is extremely significant, Alcoholic extract is significant.

4. Discussion

In the present study the preliminary phytochemical screening was studied in broad sense to explore its chemical nature, it reveals the presence of considerable amount of alkaloid, steroid, phenolic substances and vitamin C (Ascorbic acid), moderately saponins and carbohydrates, trace amount of glycoside and resins were explored from the phytochemical screening. Anti inflammatory activity of its various extracts were

performed to explore its bioefficiency. The study was took HRBC membrane stabilization method for screening of activity[9,10]. The results revealed the methanolic extract of *J. gossypifolia* showed percentage lysis of 19.1% for 100 mg/mL followed by 1.6% for 200 mg/mL of the methanolic extract. Where as aqueous extract showed 14.2% for 100 mg/mL and 56.8% for 200 mg/mL. Hence this study concludes methyanolic extrac of *J. gossypifolia* produced significant activity (membrane protection) tabulated in Table 1. The anti inflammatory activity may be due to alkaloid or steroid present in the alcoholic and aqueous extract of *J. gossypifolia*.

Conflict of interest

The authors declare they have no conflict of interests.

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